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Subphenotypes of nonsteroidal antiinflammatory disease-exacerbated respiratory disease identified by latent class analysis

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Abstract

Background: Induced sputum (IS) allows to measure mediators of asthmatic inflammation in bronchial secretions. NSAID-exacerbated respiratory disease (NERD) is recognized as a distinct asthma phenotype, usually with a severe course, eosinophilic airway inflammation, and increased production of pro-inflammatory eicosanoids. A more insightful analysis of NERD patients has shown this phenotype to be nonhomogeneous.

Objective: We aimed to identify possible subphenotypes in a cohort of NERD patients with the means of latent class analysis (LCA).

Methods: A total of 95 asthma patients with aspirin hypersensitivity underwent sputum induction. High-performance liquid chromatography or gas chromatography coupled with mass spectrometry was used to profile eicosanoids in induced sputum supernatant (ISS). Sixteen variables covering clinical characteristics, IS inflammatory cells, and eicosanoids were considered in the LCA.

Results: Three classes (subphenotypes) were distinguished within the NERD cohort. Class 1 subjects had mild-to-moderate asthma, an almost equal distribution of inflammatory cell patterns, the lowest concentrations of eicosanoids, and $\log LTE_4/\log PGE_2$ ratio. Class 2 represented severe asthma with impaired lung function despite high doses of steroids. High sputum eosinophilia was in line with higher pro-inflammatory LTE_4 in ISS and the highest $\log LTE_4/\log PGE_2$ ratio. Class 3 subjects had mild-to-moderate asthma and were also characterized by eosinophilic airway inflammation, yet increased production of pro- (LTE_4, PGD_2 and 11-dehydro-TBX_2) was balanced by

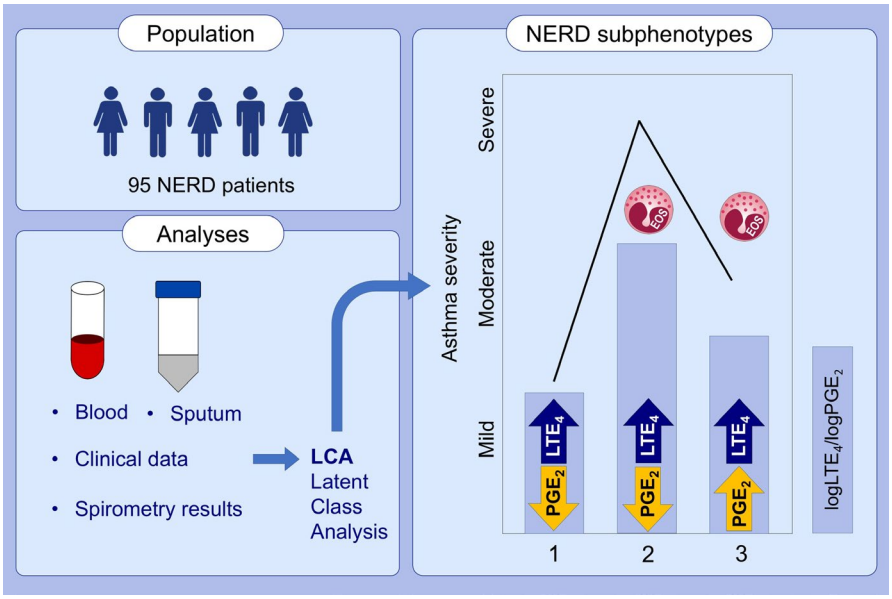
Abbreviations: 5-HETE, 5-hydroxyeicosatetraenoic acid; 11-dehydro-TBX_2, 11-dehydro-thromboxane B_2; 12-HETE, 12-hydroxyeicosatetraenoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; AA, Arachidonic acid; ACT, Asthma Control Test; AIC, Akaike information criterion; COX, Cyclooxygenase; CRS, Chronic rhinosinusitis; cysLT, Cysteinyl leukotriene; GC-MS, Gas chromatography/mass spectrometry; GLM, General linear model; HPLC-MS/MS, High-performance liquid chromatography/tandem mass spectrometry; ICS, inhaled corticosteroids; IL, Interleukin; ILC2, Innate lymphoid cell type 2; INF-γ, Interferon γ; IS, Induced sputum; ISS, Induced sputum supernatant; LCA, Latent class analysis; LLOQ, Lowest limit of quantification; LO, Lipoxygenase; LTB_4, Leukotriene B_4; LTD_4, Leukotriene D_4; LTE_4, Leukotriene E_4; NERD, NSAID-exacerbated respiratory disease; NSAIDs, Nonsteroidal anti-inflammatory drugs; OCS, oral corticosteroids; PGA_2, Prostaglandin A_2; PGD_2, Prostaglandin D_2; PGE_2, Prostaglandin E_2; uLTE_4, Urinary leukotriene E_4.

anti-inflammatory PGE_2. The value of $logLTE_4/logPGE_2$ was between values calculated for classes 1 and 3, similarly to disease control and severity.

Conclusions: LCA revealed three distinct NERD subphenotypes. Our results support a more complex pathobiology of aspirin hypersensitivity. Considering NERD heterogeneity, the relationship between inflammatory pathways and clinical manifestations of asthma may lead to more individualized treatment in difficult to treat patients in the future.

KEYWORDS

eicosanoids, induced sputum, latent class analysis, NSAID-exacerbated respiratory disease, phenotype



GRAPHICAL ABSTRACT

Heterogeneity of NERD phenotype reflects differences in inflammatory response measured by airway cells and eicosanoids. Identifying subphenotypes provides a more insightful perception and suggests a need for more individualized approach. In aspect of logLTE4/logPGE2 ratio, latent class analysis assigned subject to different groups better than identification by disease severity or control emphasizing the heterogeneity in NERD subgroup.

Abbreviations: LTE4, Leukotriene E4; NERD, NSAID-exacerbated respiratory disease; PGE2, Prostaglandin E2

1 | INTRODUCTION

According to the current recommendations nonsteroidal anti-inflammatory drugs (NSAIDs)-exacerbated respiratory disease (abbreviated as NERD) is a proper term to describe the distinct phenotype of aspirin (acetyl salicylic acid; ASA) and other NSAIDs hypersensitivity, which involves upper and lower airway mucosa.[1]

It is characterized by eosinophilic inflammation of upper and lower airways, an onset in early adulthood and a predilection for females. Respiratory symptoms are exacerbated by intake of aspirin and other nonsteroidal anti-inflammatory drugs (NSAID's).[2] Diagnosis is confirmed by aspirin challenge tests.[3] NERD usually has a severe course accompanied by chronic rhinosinusitis with recurrent nasal polyps, often requiring multiple sinus surgeries.[2,4] Atopy is rather frequent in this population.

A recent meta-analysis has indicated that 7% of asthmatics are aspirin-sensitive but that the prevalence doubles in severe asthmatics.[5] A more insightful analysis of individual patients has shown this group to be nonhomogeneous.[6,7]

There is a consensus on the nonallergic mechanism responsible for asthmatic attacks in NERD, involving abnormal metabolism of arachidonic acid (AA) by cyclooxygenase (COX) and lipoxygenase (LO) pathways.[2] Following ingestion of NSAIDs, COX-1 inhibition decreases biosynthesis of PGE_2 leading to enhanced or disinhibited synthesis of cysteinyl leukotrienes (cysLTs). CysLTs released from eosinophils and mast cells cause abrupt onset moderate-to-severe bronchoconstriction and sometimes extrapulmonary signs of facial edema and skin erythema.[2] Transcellular metabolism of AA derivatives in the airway during multi-cellular interaction generates various eicosanoids able to regulate inflammation and functional responses. The broken balance between anti-inflammatory mediators known as lipoxins,[8,9] PGE_2,[10,11] and potentially pro-inflammatory mediators such as cysLTs and PGD_2[12] has been linked to both severe asthma and NERD.[13]

The fundamental factors in NERD contributing to the underlying dysregulation of eicosanoid metabolism remain in a focus of active investigation, suggesting participation of both Th1 and Th2 cytokines.[14] More attention is paid to group 2 innate lymphoid cells (ILC2s), a lineage negative lymphocyte population that produces type 2 cytokines in allergic diseases, which are activated by mediators like cysLTs and PGD_2 overproduced in NERD.[15] Platelet-leukocyte aggregates are also thought to take part in cysLTs generation during reactions to aspirin.[16]

Asthma is now being defined in terms of phenotypes and endotypes.[17] Our everyday clinical practice supported by previous studies[6,7] shows that NERD patients present a wide spectrum of disease course yet share common features as chronic rhinosinusitis and intolerance of aspirin and other COX-1 inhibitors. Primarily defined by a pharmacological intolerance of COX-1 inhibitors, this complex endophenotype of NERD does not show clear associations with clinical measurements or laboratory biomarkers of asthma. We hypothesized that detailed characteristics, including measurement of inflammatory biomarkers in airway secretions, can provide a wider and more insightful perspective on asthma heterogeneity within this phenotype.[18]

2 | METHODS

2.1 | Subjects studied

In total, 95 patients with NERD were enrolled to the study. NSAID hypersensitivity diagnosis was established before the study based on a typical history confirmed by oral or bronchial provocation test with aspirin.[3] The asthma diagnosis was ascertained according to GINA 2018 update.[19] Patients remained without any asthma exacerbations or respiratory tract infections during the 6 weeks preceding the study and received asthma medications as currently prescribed (excluding antileukotriene drugs). None of the subjects with NERD underwent aspirin desensitization. The patients gave informed consent to participate in the study, and official approval for the study protocol from the Jagiellonian University Ethics Committee was obtained (KBET/7/B/2010). Clinical characteristics of study subjects are presented in Table 1.

2.2 | Data collection

A structured questionnaire was used to collect demographic data and detailed medical history including information on exacerbations and respiratory tract infections. The current level of asthma severity was ascertained according to GINA 2018 update.[19] Asthma control was assessed by using the Asthma Control Test (ACT). Standard spirometry and skin prick tests were performed. Blood eosinophilia and total IgE levels were measured.

Study subjects underwent sputum induction. Induced sputum was collected and processed according to ERS recommendations.[20] Induced sputum samples were investigated for sputum differential cell count and induced sputum supernatant concentrations of selected eicosanoids.

Differential count of sputum-induced cells allowed for classification into inflammatory patterns: eosinophilic—defined as induced sputum eosinophilia above 2%; neutrophilic—defined by induced sputum neutrophilia above 50%, mixed cellular with sputum eosinophilia above 2% and neutrophilia above 50%; and paucigranulocytic—when no predominant inflammation pattern was present.[20,21]

The concentration of eicosanoids in induced sputum supernatant was measured by gas chromatography/mass spectrometry (GC-MS) for PGE_2 and PGD_2, and by high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) for 5-HETE, 12-HETE, 15-HETE, LTB_4, D_4, E_4, PGA_2, and 11-dehydro-TBX_2. These analytical method details were presented elsewhere.[13]

2.3 | Analytic strategy and selection of variables

Latent class analysis considering 16 variables covering clinical characteristics such as sex, BMI, age at asthma onset, asthma control and severity, use of inhaled and/or oral steroids, history of asthma exacerbations, FEV_1, blood eosinophil count, total serum IgE, atopy status determined by skin prick tests, induced sputum inflammatory cells and selected eicosanoids—PGD_2, PGE_2, and LTE_4, was applied on the collected data set. Based on our previous studies on asthma heterogeneity, we included in the analysis lipid mediators which play a key role in aspirin hypersensitivity and have a pro-inflammatory (PGD_2 and LTE_4) or anti-inflammatory (PGE_2) impact on airways.[7]

We used LCA approach as a model-based clustering method of objects described mainly by polytomous variables supported by quantitative covariates. The latent class model seeks to stratify the cross-classification table of observed (or "manifest") variables by an unobserved ("latent") unordered categorical variable that eliminates all confounding between the manifest variable. The main assumption of the LCA model is "conditional" or "local" independence, that is, conditional upon values of latent variable responses to all of the manifest variable are assumed to be statistically independent. Covariates are included in our model through their effects on the prior probabilities of latent class membership. Our model allows individuals' prior so vary depending upon their observed covariates.

The R package poLCA[22] was used for estimating parameters of LCA models and for identification of the optimal model according to AIC (Akaike's information criterion) in a stepwise manner by the analysis of models with the number of classes starting from 1 to 4 (for 5 classes model number of parameters estimated (96) exceeds number of observations (95)).

AIC is founded on information theory and deals with the trade-off between the goodness-of-fit of the model and the complexity of the model. More information about AIC can be found in http://en.wikipedia.org/wiki/Akaike_information_criterion. Each subject was allocated when the best model was determined.

TABLE 1 Characteristics of the entire NERD cohort

Variable	Value
No. of study subjects	95
Female/male subjects, no. (%)	68 (71.6)/27 (28.4)
Age (y), mean ± SD	46.6 ± 12.5
Age at asthma onset (y), mean ± SD	32.6 ± 14.9
Asthma onset > 12 y, no. (%), yes/no	93 (97.9)/2 (2.1)
Duration of asthma (y), median (25th-75th pc)	10 (6-19)
BMI, mean ± SD	26.9 (5.2)
>30 kg/m^2, no. (%), yes/no	21 (22.1)/74 (77.9)
ACT mean ± SD	21 ± 4.4
Present level of asthma control (ACT), no. (%)	
1: Well controlled	39 (41.1)
2: Partially controlled	35 (36.8)
3: Uncontrolled	21 (22.1)
FEV1 (% predicted), mean ± SD	88.5 ± 16
Exacerbations, median (25th-75th pc)	1 (0-2)
Upper airway symptoms (CRS), no. (%), yes/no	95 (100)/0 (0)
CRS surgery, no. (%), yes/no	66 (69.5)/25 (30.5)
Nasal polyps, no. (%), yes/no	81 (85.3)/14 (14.7)
Positive skin prick test responses, no. (%), yes/no	35 (36.8)/60 (63.2)
IgE total, median (25th-75th pc)	102.5 (32.6-216)
Blood eosinophil count, median (25th-75th pc)	389.5 (240-625)
Sputum eosinophilia,%, median (25th-75th pc)	3.7 (0.7-14.6)
Levels of asthma severity, no. (%)	
1:Intermittent	9 (9.5)
2:Mild to moderate persistent	48 (50.5)
3:Severe persistent	38 (40)
Treatment of asthma	
OCS, no. (%), yes/no	6 (6.3)/89 (93.7)
OCS (mg/d) methylprednisolone, median (25th-75th pc)	4 (4-8)
ICS, no. (%), yes/no	86 (90.5)/9 (9.5)
ICS (μg/d) fluticasone, median (25th-75th pc)	500 (200-1000)

Abbreviations: ACT, Asthma Control Test; BMI, body mass index; CRS, chronic rhinosinusitis; ICS, inhaled corticosteroids; OCS, oral corticosteroids.

2.4 | Statistical analysis

Summary statistics were presented as mean, standard deviation, median, 25th and 75th percentiles and number in each category with percentage of total. We transformed mediator concentrations to their logarithm or used Box-Cox transformation to normalize the distributions before analysis. Normality was checked using the Shapiro-Wilk test. A general linear model (GLM) and a post hoc Tukey's test were applied to analyze the differences in mediator levels between latent classes. Categorical data were analyzed using the chi-square and Fisher exact tests. A $P < .05$ was considered statistically significant. All other statistical analyses were performed with the use of Dell Statistica (v.13) and Origin Pro(v.9.1).

3 | RESULTS

The clinical characteristics for the entire NERD cohort are presented in Table 1. The main characteristics of the respective classes are summarized below in Tables 2 and 3; and Figures 1 and 2.

Forty-three patients (45.3%) were allocated to class 1, which was defined as having "mild-to-moderate asthma course, with no lung impairment, low sputum eosinophilia and the lowest concentrations of mediators in induced sputum." Patients in this class were predominantly female 70%, with a mean age 46.5 ± 11.5y. This class was characterized by an almost equal prevalence of inflammatory phenotypes based on induced sputum cell counts—that is 30% of subjects had eosinophilic, 30% neutrophilic, 30% paucigranulocytic, and 10% mixed eosinophilic and neutrophilic pattern. Sputum eosinophilia (median 1.1%) was significantly lower than in classes 2 ($P = .004$) and 3 ($P = .03$). Asthma tended to be mild-to-moderate (63%), not rarely intermittent (19%), without lung function impairment (mean FEV_1 96% ±9.8%). None of the subjects had uncontrolled asthma (mean ACT score 23.4 ± 2.3). Patients scarcely experienced exacerbations (median 0 per year). Asthma control was achieved by administration of low doses (median 250 mcg of fluticasone daily) of inhaled corticosteroids (ICS). About 39.5% of asthmatics were atopic. The concentration of LTE_4 in ISS was lower compared with classes 2 ($P = .003$) and 3 ($P < .001$), while concentrations of PGD_2, PGE_2, and 11-dehydro-TBX_2 were lower than in class 3 ($P < .001$ for above mentioned).

Thirty-six subjects (37.9%) were assigned to class 2, which was defined by asthma with a severe course, poorly controlled, with airway obstruction, frequent exacerbations, eosinophilic airway inflammation, and increased pro-inflammatory mediators in induced sputum. Similarly to class 1, subjects were predominantly women (83%), with a mean age of 48.3 ± 13.3y. The induced sputum inflammatory patterns were mainly eosinophilic (64%) and mixed eosinophilic and neutrophilic (14%). In comparison with class 1, this class was characterized by high sputum eosinophilia (median 9.6%). Eosinophilic inflammation was accompanied by more severe course of asthma with lower FEV_1 values (mean 78.8% ±18). Severe asthma was diagnosed in 69%, mild and moderate in 31% of study subjects, while no one had intermittent asthma. Asthma remained uncontrolled in 53% of patients, mean ACT result was 18.4 ± 4.4. More intense treatment was needed, as all subjects received ICS (median 1000 mcg of fluticasone daily), and 20% of subjects required oral corticosteroid (OCS) treatment. The number of exacerbations (median 1 per year) was higher than in class 1. 33% of asthmatics were atopic. LTE_4 in ISS was significantly higher compared with class 1 ($P < .003$), but lower than in class 3 ($P = .019$). Concentrations of PGD_2 ($P = .001$), PGA_2 ($P = .007$), PGE_2 ($P = .007$), 5-HETE ($P = .006$), and 11-dehydro-TBX_2 ($P < .001$) were lower than in class 3.

TABLE 2 Clinical characteristics of the particular classes

	Class 1 (n = 43)	Class 2 (n = 36)	Class 3 (n = 16)	*P*
Female/male subjects, no. (%)	30 (69.8)/13 (29.2)	30 (83.3)/6 (6.7) [3]	8 (50)/8 (50) [2]	.046
Age (y), mean ± SD	44.7 ± 11.5	48.3 ± 13.5	48.1 ± 12.6	NS
Age at asthma onset (y), mean ± SD	33.2 ± 17.5	30.2 ± 15.2	33.2 ± 13.8	NS
Asthma onset > 12 y, no. (%), yes/no	42 (97.7)/1 (2.3)	36 (100)/0 (0)	15 (93.8)/1 (6.2)	NS
Duration of asthma (y), median (25th-75th pc)	7 (5-16)	13 (9-23)	10 (7-21)	NS
BMI, mean ± SD	26.3 ± 4	26.4 ± 5.5	29.8 ± 6.4	NS
>30 kg/m^2, no. (%), yes/no	5 (11.6)/38 (88.4) [3]	9 (33.3)/27 (66.7)	7 (43.8)/9 (56.2)[1]	.026
ACT mean ± SD	23.4 ± 2.3 [2,3]	18.4 ± 4.4 [1]	20.6 ± 5 [1]	<.001
Present level of asthma control (ACT), no. (%)				
1: Well controlled	31 (72.1) [2,3]	3 (8.3) [1,3]	5 (31.3) [1,2]	<.001
2: Partially controlled	12 (27.9) [3]	14 (38.9)	9 (56.3)[1]	<.001
3: Uncontrolled	0 (0) [2,3]	19 (52.8) [1,3]	2 (12.4) [2,3]	<.001
FEV1 (% predicted), mean ± SD	96 ± 9.8 [2]	78.8 ± 18 [1,3]	89.7 ± 14.2 [1]	<.001
Exacerbations, median (25th-75th pc)	0 (0-1)[2]	1 (1-4)[1]	0 (0-2)	.002
Upper airway symptoms (CRS), no. (%), yes/no	43 (100)/0 (0)	36 (100)/0 (0)	16 (100)/0 (0)	NS
CRS surgery, no. (%), yes/no	32 (74.4)/11 (24.6)	26 (72.3)/10 (27.7)	8 (50)/8 (50)	NS
Nasal polyps, no. (%), yes/no	35 (81.4)/8 (18.6)	32 (88.9)/4 (11.1)	14 (87.5)/2 (12.5)	NS
Positive skin prick test responses, no. (%), yes/no	17 (39.5)/26 (60.5)	12 (33.3)/24 (66.7)	6 (62.5)/10 (32.5)	NS
IgE total, median (25th-75th pc)	54.5 (24.1-141) [3]	122 (37-318)	194.5 (136-268) [1]	.003
Blood eosinophil count, median (25th-75th pc)	373 (240-376)	375 (252-743)	510 (200-612)	NS
Sputum eosinophilia, %, median (25th-75th pc)	1.1 (0.4-6.4) [2,3]	9.6 (3.6-19.9) [1]	9.2 (2.5-20.5) [1]	.002
Levels of asthma severity, no. (%)				
1: Intermittent	8 (18.6)[2]	0 [1]	1 (6.3)	<.001
2: Mild-to-moderate persistent	27 (62.8)[2]	11 (30.6)[1,3]	10 (62.5)[2]	<.001
3: Severe persistent	8 (18.6)[2]	25 (69.4)[1,3]	5 (31.3) [2]	<.001
Treatment of asthma				
OCS, no. (%), yes/no	0 (0)/43 (100)[2]	6 (16.7) [1]	0 (0)/16 (100)	.048
OCS (mg/d) methylprednisolone, median (25th-75th pc)	0	4 (4-8)	0	<.001
ICS, no. (%), yes/no	35 (81.4)/8 (18.6)	36 (100)/0 (0)	15 (93.8)/1 (6.2)	.056
ICS (μg/d) fluticasone, median (25th-75th pc)	250 (100-500)[2]	1000 (500-1000)[1,3]	320 (100-500)[2]	<.001

Abbreviations: ACT, Asthma Control Test; BMI, body mass index; CRS, chronic rhinosinusitis; ICS, inhaled corticosteroids; OCS, oral corticosteroids. Indices 1-3 show statistical significant difference between two classes (pairwise comparisons).

Sixteen patients (16.8%) were grouped into class 3, which was characterized by asthma with a mild-to-moderate course, relatively well controlled, with eosinophilic airway inflammation and increased both pro- and anti-inflammatory mediators in induced sputum. This group was equally comprised of women and men with a mean age of 48.1 ± 12.6y. Obesity was quite frequent in this class (78%). The induced sputum specimens were mainly eosinophilic (50%) and mixed eosinophilic and neutrophilic (38%). This class was also characterized by high sputum eosinophilia (median 9.2%). No significant differences were observed in blood eosinophilia between neither classes. The course of asthma was most often mild-to-moderate (63%), but with lower FEV_1 (89.7% ±14.2%) values compared with class 1 (P = .03). Asthma was most frequently partially controlled (56%) with mean ACT score 20.6 ± 5. Almost all subjects were treated with inhaled corticosteroids (median 320 mcg of fluticasone daily). 60% of subjects were atopic, with the highest total serum IgE levels. In this class, compared with classes 1 and 2, significantly higher concentrations of pro-inflammatory lipid mediators as LTE_4 (P < .001 and P = .019, respectively), PGD_2 (P < 001 and P = .01, respectively), 11-dehydro-TBX_2, (P < .001 and P < .001, respectively) and higher concentration of anti-inflammatory PGE_2 (P < .001 and P < .001, respectively) were observed. Concentrations of PGA_2 (P = .007) and 5-HETE (P = .006) were higher compared to class 2.

3.1 | Pro- and anti-inflammatory eicosanoids

The between-classes analysis of single mediator concentrations did not correlate with asthma severity and disease control; furthermore, the concentration of pro- and anti-inflammatory arachidonic acid

TABLE 3 Concentrations of selected eicosanoids in induced sputum supernatant in particular classes

(pg/ml), median (25th-75th percentile) Metabolite, method, LLOQ	Class 1 (n = 43)	Class 2 (n = 36)	Class 3 (n = 16)	*P*
LTE_4 HPLC-MS/MS 3.22	56.2 (10.1-64.5) [2,3]	125 (36.1-131.9) [1,3]	305.4 (126.4-446.7) [1,2]	<.001
PGE_2 GC-MS 1.62	73.6 (43.5-87.9)[3]	60.6 (32.4-74.2)[3]	227.3 (93.6-289.6)[1,2]	<.001
LTB_4 HPLC-MS/MS 2.96	1039.2 (209.6-1606)	477 (172.2-528.5)	1224.5 (332.2-1669.9)	.03
PGA_2 HPLC-MS/MS 12	30.2 (12.8-38.6)	20.2 (11.7-27.7) [3]	48 (22.7-50.3) [2]	.007
PGD_2 GC-MS 2.09	44.5 (15.7-55.9) [3]	43.8 (16.7-66.4) [3]	104.9 (65.2-130.4) [1,2]	<.001
5-HETE HPLC-MS/MS 0.82	1233.7 (338.9-1866.3)	699.6 (235.1-1099.3) [3]	1941.8 (7360-3004.6) [2]	.007
12-HETE HPLC-MS/MS 0.63	1285.8 (667-2480.3)	924.8 (652.1-1662.8)	1331.4 (492.8-3315.5)	NS
15-HETE HPLC-MS/MS 2.18	715.6 (317.5-1704.9)	643.4 (354.1-1712.8)	1336.8 (539.9-4830.4)	NS
11-dehydro-TBX_2 HPLC-MS/MS 1.84	19.4 (15.2-20,8) [3]	31.9 (16.5-22.9)[3]	41.4 (23.2-89.7)[1,2]	<.001

Abbreviations: GC-MS, gas chromatography/mass spectrometry; HPLC-MS/MS, high-performance liquid chromatography/tandem mass spectrometry; LLOQ, lowest limit of quantification.

Indices 1-3 show statistical significant difference between two classes (pairwise comparisons).

metabolites was significantly higher in class 3 than in classes 1 and 2. Thus, we looked at the concentrations ratio of pro- and anti-inflammatory mediators that are well known to be important in the pathogenesis of NERD[2]—$logLTE_4/logPGE_2$ (Figure2) vs. asthma control and severity. We found that this ratio differs significantly between class 1 (0.8) and classes 2 (1.2) and 3 (1.0), no statistical significance was observed between classes 2 and 3. Values of $logLTE_4/logPGE_2$ ratio follow the clinical course of asthma (Tables 1 and 2). The lowest ratio was found in class 1 with good asthma control and milder disease, while the highest ratio was observed in class 2 characterized by worse asthma control and high grade of asthma severity. In class 3, the value of $logLTE_4/logPGE_2$ ratio was between values calculated for classes 1 and 3, similarly to disease control and severity. There was a positive correlation between sputum eosinophilia and concentrations of LTE_4 ($r = 0.58$, $P < .001$) and PGD_2 ($r = 0.38$, $P = .001$) in ISS. LTE_4 concentration also correlated positively with PGD_2 concentration measured in ISS ($r = 0.47$, $P < .001$). No significant correlations were found between PGE_2 and both PGD_2 and LTE_4 for the whole NERD group, with a negative correlation between LTE_4 and PGE_2 only in class 2 ($r=-0.5$, $P = .048$).

4 | DISCUSSION

The application of latent class analysis revealed three clearly defined classes among the NERD cohort. This grouped together aspirin hypersensitive asthmatics according to similarities of specific asthma features, induced sputum cell count and lipids mediators measured in ISS (each variable is statistically independent). Class 1 represented well-controlled mild-to-moderate asthma, with low sputum eosinophilia, no clear indication for a dominant inflammation pattern in the bronchial airways and lower concentrations of eicosanoids in IS. However, subjects in class 2 had dominantly severe, poorly controlled eosinophilic asthma. High sputum eosinophilia correlated with the highest concentration of LTE_4 in ISS. Class 3 also represented eosinophilic asthma but with a milder course and better lung function. It is interesting that there was no statistical difference in $logLTE_4/logPGE_2$ ratio when we compared our subjects in groups defined only by asthma severity or asthma control (data not shown), that is, in the aspect of $logLTE_4/logPGE_2$ ratio LCA assigned subjects to different groups better than only identification by

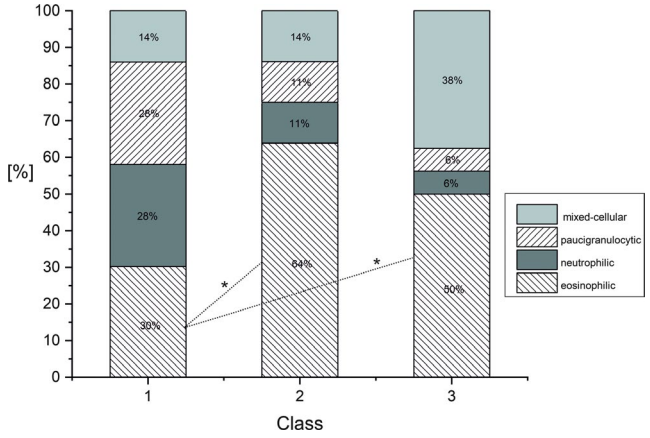


FIGURE 1 Distribution of inflammatory phenotypes based on induced sputum cell count in three latent classes (*-significant difference, $P < .05$ between class)

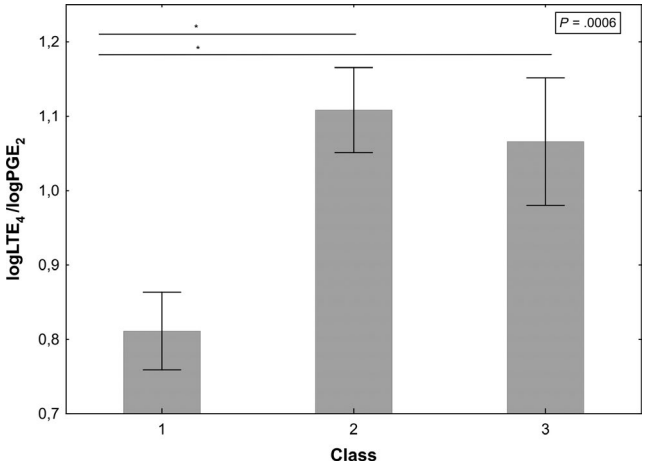


FIGURE 2 LogLTE$_4$/logPGE$_2$ ratio in three latent classes, (*-significant difference, $P < .05$ between class)

disease severity or control. Thus, this study presents an approach integrating clinical data and laboratory measures of inflammation to describe NERD heterogeneity. The LCA was previously applied to identify possible NERD subphenotypes in a study by Bochenek et al including only urinary LTE$_4$ (uLTE$_4$) as an inflammatory biomarker.[6] Four classes with disease severity ranging from mild to severe with frequent exacerbations and high healthcare utilization were established. In this study, the highest levels of uLTE$_4$ among NERD were observed among subjects within classes of moderate course, intensive upper airway symptoms, and high blood eosinophil levels. There were some similarities and differences to our previous observations regarding NERD heterogeneity,[7] possibly because of the limited number of subjects taken into analysis not covering all possible NERD subphenotypes. Both studies identified two similar groups of asthmatics: one with severe disease, impaired lung function, eosinophilic inflammation and increased LTE$_4$ in ISS and second with mild-to-moderate asthma, no predominant inflammatory pattern and low LTE$_4$ in ISS. The main difference of the present study is identification of subgroup of NERD subjects with eosinophilic inflammation, relatively good asthma control, better lung function, and both increased pro- and anti-inflammatory mediators in ISS.

NERD is usually described as moderate-to-severe asthma requiring ICS and not rarely systemic corticosteroid to achieve disease control.[2] We found in this study that patients with the most severe course of asthma were grouped together in class 2. It was characterized by lack of asthma control despite high doses of ICS, need for OCS, lung function impairment, and a presence of disease exacerbations. Therefore, this class resembled the most a typical clinical presentation of NERD previously described in literature.[2]

Asthma is currently thought to consist of multiple phenotypes rather than a single disease entity. Asthma phenotypes were initially focused on combinations of clinical characteristics, but the concept evolved to link biology to phenotype, often through a statistically based process. This idea of adult asthma phenotypes was described by Wenzel in Nature in 2012, grouping asthmatics into five phenotypes: early-onset allergic, late-onset eosinophilic, exercise-induced, neutrophilic, and obesity-related.[23] NERD was recognized as a subphenotype of late-onset eosinophilic asthma. Persistent eosinophilic inflammation of the airways despite corticosteroid therapy was associated with an adult-onset, less frequent allergic sensitization, often with concurrent chronic sinusitis, nasal polyps, and sometimes NERD.[23-25] Late-onset eosinophilic phenotype was linked with Th2 inflammatory process. The cysteinyl leukotriene pathway in NERD is upregulated by Th2 cytokines, and these potent pro-inflammatory lipid mediators are released from cells associated with Th2 inflammation like eosinophils, basophils, and mast cells.[23,24]

The degree of overlap between late-onset eosinophilic asthma with NERD and late-onset eosinophilic asthma without NERD is not clear. It has also been shown by means of LCA that eosinophilic inflammation, intense upper and lower airway symptoms, overproduction of cysLTs, and PGD$_2$ can characterize both aspirin-tolerant and hypersensitive asthma.[7]

Eosinophilic asthma includes either allergic or nonallergic phenotypes underlying immune responses mediated by T helper Th2 cell-derived cytokines. Eosinophilic inflammation can be associated with the whole spectrum of asthma severity, ranging from mild-to-moderate to severe disease and are associated with worse control and disease exacerbations.[25,26] More severe disease is frequently accompanied by mixed patterns of inflammation including both eosinophils and neutrophils. Neutrophilic airway inflammation is thought to be triggered by Th1 and especially by Th17 lymphocytes. Neutrophils often represent the predominant inflammatory cells detected in the induced sputum obtained from patients with severely obstructed airways, experiencing uncontrolled symptoms and frequent exacerbations despite high intensity of treatment.[23,25,27] Additionally, neutrophils generated more reactive oxygen species in patients with AERD and in those with severe asthma than in those with ATA or nonsevere asthma.[28] In this study, all inflammatory patterns were seen in the NERD cohort. Classes 2 and 3 that differed in the course of asthma were characterized by higher sputum eosinophilia. These observations are line with results of studies

including whole asthmatic populations showing eosinophilic asthma to be very nonhomogeneous.[7,25]

The pathogenesis of NERD still remains largely elusive despite years of studies. Initiation and persistence of inflammation are strongly dependent on type 2 cytokines, eosinophils, mast cells, cysLTs, and PGD_2. CysLTs like LTE_4 that are overproduced in classes 2 and 3 are strong mediators of allergic inflammation mainly released by eosinophils and mast cells.[29] In numerous studies, the key role of cysLTs overproduction in NERD was well documented on the systemic level and increased urinary excretion of LTE_4 as a hallmark of this disease.[2,30] Increased local and systemic biosynthesis of pro-inflammatory PGD_2 and its metabolites was also found inaspirin hypersensitive asthmatics.[12(p2)] PGE_2 plays a protective role in the lungs. It exerts a bronchodilatory effect through a direct effect on the smooth muscle of the bronchi, but also has anti-inflammatory activity, reducing the release of mediators by mast cells and eosinophils.[31] In class 2 concentration of bronchoprotective PGE_2 in ISS was the lowest, which could possibly explain the worse lung function, severe course of the disease and frequent exacerbations, whereas class 3 was characterized by the highest concentration of PGE_2 in ISS. The milder course of asthma in this class despite eosinophilic inflammation and increased concentration of pro-inflammatory mediators could possibly be explained by the rescue effect of this prostanoid on airways.

We also analyzed the ratio of $logLTE_4/logPGE_2$ mediators between classes to look for the best parameter describing clinical course and because the concentration of pro- and anti-inflammatory arachidonic acid metabolites in class 3 was significantly higher than in classes 1 and 2. Statistical analysis of this ratio revealed significant differences between class 1 vs classes 2 and 3—consistent with asthma control and severity in classes, showing that the overbalance of PGE_2 vs. LTE_4 influences the disease course in classes defined by LCA.

Most studies in NERD have shown a cytokine profile consistent with type 2 immunity; however, some studies suggest a possible role for Th1 response and IFN-γ as well.[32,33] Both IL-4 and IFN-γ increase LTC_4 synthase expression on mast cells and eosinophils, providing a link for the upregulation of CysLTs seen in NERD.[34] Eosinophils and mast cells expressing 5-LO and LTC_4 synthase are the main source of cysLTs in hypersensitivity reactions, yet platelets may also play a role in cysLTs overproduction. Platelet-leukocyte aggregates were found in higher concentrations in blood and upper airway tissue from NERD patients reflecting systemic overproduction of cysLTs measured by urinary LTE_4.[16] Other important cells in NSAIDs provoked reactions are the ILC2s present in lower and upper airways.[35-37] ILC2s activation is dependent on local respiratory epithelium damage and results in the generation of pro-inflammatory cytokines and enhancement of upper and lower inflammation, mucus production, bronchoconstriction and remodeling. It was shown that ILC2s are recruited to nasal mucosa of NERD patients after COX-1 inhibitor administration, correlating with enhanced production of prostaglandins and leukotrienes.[15,38] Concurrently, ILC2s activate and recruit mast cells and eosinophils[39] by production of cytokines such as IL-5 and IL-9 but in a response to released PGD_2 and cysLTs ILC2s are activated. These observations show how complex the pathogenesis of aspirin exacerbated disease is, including not only the alternations in eicosanoid production after COX-1 inhibition. In our study, we have two eosinophilic asthma classes but differing in clinical course and concentrations of eicosanoids measured in ISS. It is possible that more than one pathway in NERD depending on the local environment can lead to eosinophilic inflammation with a different eicosanoid profile, reflecting the heterogeneity of this asthma phenotype.

In our study, LCA revealed subphenotypes within NERD cohort, which is by itself regarded as a distinct asthma phenotype. We used a model based on 16 variables including clinical data, inflammatory cell count, and eicosanoids measured in ISS to address different aspects of the disease including underlying biochemical processes. The model with three latent classes was selected as the best one fitting the analyzed data basing on AIC. Furthermore, the high values of posterior probability indicated that participants were assigned to classes in an unambiguous manner.

We are aware of limitations of our study. It might be questioned if such a stratification of the NERD cohort into classes is a realistic approach. The distribution of patients may change depending on population size and selected variables. It is known that inflammatory patterns of influx cells in the airways may change over time, as many factors such as administration of corticosteroids or exposition to allergens influence the composition of influx cells[40-42] on inflammatory cell subtypes in asthma.

It must be stated that the stability of subphenotypes over time has not been analyzed.

More data from recent studies support the idea of a more complex pathogenesis of NERD. Our experience with NERD patients suggests the need to reconsider NSAID hypersensitive asthma in means of subphenotypes requiring a different therapeutical approach.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NCW, KW, AC, MS, and LM contributed to project concept, study design, and study implementation; NCW, KW, PN, AK, and KT contributed to data collection; NCW, PN, and AC contributed to data and statistical analysis; NCW, KW, MS, AC, and LM contributed to writing of the first draft of the manuscript; all authors contributed to manuscript editing; all authors reviewed and approved the final version of the manuscript.

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